

## *In vitro* activity of linezolid & quinupristin/dalfopristin against Gram-positive cocci

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**Background & objectives:** Since the incidence of vancomycin- and methicillin-resistant Gram-positive infections continue to increase, novel antimicrobials such as linezolid and streptogramin may provide new options to treat patients. The aim of this study was to investigate *in vitro* susceptibility of *Enterococcus faecium* resistant to glycopeptides, coagulase negative staphylococci and *S. aureus* resistant to methicillin isolated mainly from blood and also rectal swab cultures of patients against quinupristin/dalfopristin and linezolid.

**Methods:** The *in vitro* susceptibility to linezolid and quinupristin/dalfopristin for a total of 332 isolates of Gram-positive cocci [127 methicillin-resistant *Staphylococcus aureus*, 109 methicillin-resistant coagulase negative staphylococci (71 *S. epidermidis*, 38 *S. haemolyticus*) and 96 *vnaA* genotype vancomycin-resistant *Enterococcus faecium*] was investigated by E test.

**Results:** All MRSA and MRCoNS isolates were susceptible to linezolid (MICs < 4.0 mg/l). Ninety per cent of VRE isolates were inhibited by linezolid at concentration of 2.0 mg/l and presented similar activities to quinupristin/dalfopristin. MICs for quinupristin/dalfopristin against staphylococci were also low (MIC<sub>90</sub> = 1.0 mg/l for both MRSA and MRCoNS isolates).

**Interpretation & conclusion:** The results of the present study demonstrated that quinupristin/dalfopristin and linezolid, have good *in vitro* activity against MRSA, MRCoNS and vancomycin resistant *E. faecium* in Turkey. These drugs could be promising therapeutic options in an era of rapidly growing antibiotic resistance in all parts of world.

**Key words** Enterococci-linezolid-methicillin resistance-quinupristin/dalfopristin-staphylococci-vancomycin resistance

The incidence of infections caused by multidrug resistant (MDR) Gram-positive bacteria is increasing despite the advances in antibacterial therapy over the last few years. The most problematic pathogens include strains of enterococci resistant to

glycopeptides, and staphylococci resistant to methicillin. These organisms are frequently resistant to the other most currently available antibacterials, and the infections are extremely difficult to treat. Thus, new antibiotics are required to treat infections caused by these MDR organisms<sup>1,2</sup>.

Quinupristin/dalfopristin is a new water soluble streptogramin antimicrobial agent comprising quinupristin and dalfopristin in a ratio of 30:70. The *in vitro* spectrum of activity includes most Gram-positive aerobes, significant Gram-negative aerobes, Gram-positive anaerobes and intracellular bacteria that are causal agents of various infections. Of particular note, quinupristin/dalfopristin is active against MDR isolates of *Staphylococcus aureus*, *S. epidermidis* and *Enterococcus faecium*. Bactericidal activity and a prolonged post-antibiotic effect have also been noted for quinupristin/dalfopristin against Gram-positive cocci. Overall, the spectrum of antibacterial activity indicates a potential role for this combination in the treatment of difficult-to-treat Gram-positive infections, including those caused by MDR organisms<sup>1,3-5</sup>.

Linezolid is also one of the newer antibacterial agents with a spectrum of activity against Gram-positive bacteria<sup>6</sup>. It is the first drug of a new class of antibiotics, the oxazolidinones. Oxazolidinones provide activity against, methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci (VRE). This class of compounds possesses a unique mechanism of action, binding to the bacterial 50S ribosomal subunit to inhibit the initiation of protein synthesis. Because of this unique mechanism, oxazolidinones do not demonstrate cross resistance with currently available antibiotics<sup>1,2,6,7</sup>.

Because the incidence of vancomycin- and methicillin-resistant Gram-positive infections continues to increase, alternative options for treatment are necessary<sup>8-10</sup>. Currently available antimicrobial agents such as linezolid and quinupristin/dalfopristin may provide new options to treat these patients. This study was undertaken to investigate the *in vitro* susceptibility of Gram-positive cocci that include enterococci resistant to vancomycin and teicoplanin, coagulase negative staphylococci and *S. aureus* resistant to methicillin, against quinupristin/dalfopristin and linezolid in order to understand the quinupristin/dalfopristin susceptibility pattern among these Gram-positive bacteria in the Aegean region of Turkey.

## Material & Methods

A total of 332 isolates of Gram-positive cocci were recovered from blood and rectal swab specimens of patients hospitalized for more than 48 h at Ege University Hospital, Izmir, Turkey from November 2002 to October 2003. Rectal swabs were screened for vancomycin resistance using brain heart infusion agar (Oxoid, England) containing vancomycin 6 mg/l, and blood cultures were performed by using Bact/Alert system (bioMérieux, France). Only one isolate per patient was included. These isolates were identified at species or genus level by conventional methods. These methods include colony morphology and haemolysis form on 5 per cent sheep blood agar, Gram's staining and morphological characterization, catalase production, glucose fermentation and coagulase reaction for staphylococci, also colony morphology and haemolysis pattern on 5 per cent sheep blood agar, Gram's staining and morphological characterization, catalase and L-pyrrolidonyl- $\beta$ -naphthylamide hydrolase (PYR) production, ability to growth in broth containing 6.5 per cent NaCl and hydrolyze esculin in the presence of 40 per cent bile salts for enterococci<sup>11,12</sup>. The following commercial identification systems the API 20 Strep system (bioMérieux, France) for streptococci, and the API Staph system for staphylococci were used to identify the strains at species level.

Methicillin resistance of isolated staphylococci was detected by using agar screening plates according to guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS)<sup>13</sup>. Enterococci were tested for glycopeptide resistance by plating on screening agar supplemented with 6 mg/l vancomycin according to NCCLS<sup>13</sup>. For all isolates that grew on this medium, minimal inhibitory concentrations (MICs) of vancomycin and teicoplanin were determined by E test (AB-Biodisk, Sweden). Detection of the resistance genotypes for enterococci were done by PCR, as described by Dutka-Malen *et al*<sup>14</sup> and all were *vanA* genotype. All MRSA, methicillin-resistant coagulase negative staphylococci (MRCoNS) and VRE isolates were also tested for antimicrobials of different chemical classes. Susceptibility testing for methicillin-resistant staphylococci against gentamicin, erythromycin,

clindamycin, ofloxacin, tetracycline and trimethoprim/sulphamethoxazole and for VRE against penicillin, gentamicin (high level), erythromycin and tetracycline was carried out by disk diffusion method and interpreted according to NCCLS breakpoints<sup>13</sup>. The vancomycin agar screen test containing brain heart infusion agar (Oxoid, England) and 6 mg/l vancomycin was also used for staphylococci for vancomycin resistance<sup>15</sup>. For staphylococci and enterococci, multidrug resistance is defined as concurrent resistance to three or more antimicrobials of different clinical classes<sup>16</sup>. The isolates were stored at -70°C in Protect Bacterial Preservers (Technical Service Consultant Limited, Lancashire, England).

To determine the activities of linezolid and quinupristin/dalfopristin, Mueller-Hinton agar (Oxoid, England) plates for enterococci and Mueller-Hinton agar plates supplemented with 2 per cent NaCl for staphylococci were inoculated by swabbing of the surface with a suspension of organisms adjusted to equal the turbidity of a 0.5 McFarland opacity standard. Inoculated plates were allowed to dry before E test strips containing linezolid (range 0.016-256 mg/l) and quinupristin/dalfopristin (range 0.002-32 mg/l) were applied onto the surface of the agar. After incubation for 22 to 24 h at 37°C in ambient air the MIC was read directly from the intersection of the inhibition ellipse with the test strip MIC scale. NCCLS breakpoints were used to interpret MIC results (quinupristin/dalfopristin MIC  $\leq$  1 mg/l as susceptible, MIC  $\geq$  4 mg/l as resistant for staphylococci and enterococci; linezolid MIC  $\leq$  2 mg/l as susceptible, MIC  $\geq$  8 mg/l as resistant for enterococci and the breakpoint for susceptibility is  $\leq$  4.0 mg/l for staphylococci<sup>13</sup>).

The following reference strains were included as controls: *S. aureus* ATCC 29213, and ATCC 43300, *E. faecalis* ATCC 29212, *E. faecalis* ATCC 51299 (Oxoid, England). Additionally, 3-5 per cent sample of Mueller-Hinton medium from each batch was incubated without any inoculation for two days at 35°C and also inoculated with *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 for sterility controls and growth performance of media.

Fischer's exact test was used to analyse the data statistically.

## Results

A total of 127 isolates of MRSA, 109 isolates of MRCoNS (71 *S. epidermidis*, 38 *S. haemolyticus*) and 96 isolates of vanA genotype VRE (all were *E. faecium* and vancomycin MICs  $>$  32 mg/l) were studied. All methicillin-resistant staphylococci were also resistant to three or more antimicrobials among aminoglycosides, lincosamides, quinolones, tetracyclines and trimethoprim/sulphamethoxazole and all the VRE isolates showed the similar resistance pattern to penicillin, gentamicin (high level), macrolides and tetracyclines. Therefore, the isolates were defined as MDR. All staphylococci were susceptible to vancomycin.

Linezolid and quinupristin/dalfopristin inhibited all isolates at concentrations between 0.25-4.0 mg/l and 0.125-3.0 mg/l, respectively. The MIC ranges, MIC<sub>50</sub> and MIC<sub>90</sub> values and susceptibility percentages for the MRSA, MRCoNS, and vancomycin-resistant *E. faecium* isolates (VREF) are shown in the Table.

Although MICs of linezolid for MRSA were slightly higher than MRCoNS MICs, all of the MRSA and MRCoNS isolates were susceptible (MIC<sub>90</sub>s, 4.0 and 2.0 mg/l, respectively). Also among the MRCoNS, *S. epidermidis* and *S. haemolyticus* showed the similar susceptibility pattern for linezolid. MICs of quinupristin/dalfopristin were two to three times lower dilutions than linezolid for all staphylococcal strains. MIC<sub>90</sub>s for quinupristin/dalfopristin were 1.0 mg/l for both MRSA and MRCoNS strains. There were no resistant isolates and only three MRSA isolates (MICs=1.5, 2.0, and 3.0 mg/l) and two MRCoNS isolates (MICs=1.5 and 3.0 mg/l) were intermediate. Overall susceptibility of 236 staphylococci were 97.9 per cent and there was no statistically significant difference between susceptibilities of MRSA (124/127, 97.6%) (percentages of resistant isolates 2.4%, 95% confidence interval-CI, 0-5.0%) and MRCoNS (107/109, 98.2%) (percentage of resistant isolates 1.8%, 95% CI, 0-4.3%) and between *S. epidermidis* (69/71, 97.2%) (percentage of resistant isolates 2.8%, 95% CI, 0-6.6%) and *S. haemolyticus* (38/38, 100%) for quinupristin/dalfopristin.

Ninety per cent of VREF with vanA phenotypes were inhibited by linezolid at concentrations of

**Table.** *In vitro* activity of linezolid and quinupristin-dalfopristin against methicillin-resistant *Staphylococcus aureus*, methicillin-resistant coagulase negative staphylococci and vancomycin-resistant *Enterococcus* isolates

Organism	Linezolid				Quinupristin-dalfopristin			
	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	S	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	S
MRSA (n=127)	0.5-4.0	2.0	4.0	100	0.38-3.0	0.75	1.0	97.6
MRCoNS (n=109)	0.25-3.0	1.5	2.0	100	0.125-3.0	0.5	1.0	98.2
MRSE (n=71)	0.25-3.0	1.5	2.0	100	0.125-2.0	0.5	1.0	97.2
MRSH (n=38)	0.38-3.0	1.5	2.0	100	0.25-3.0	0.5	1.0	100
VREF (n=96)	0.75-4.0	1.5	2.0	94.8	0.38-2.0	0.75	1.5	94.8

50% and 90%, MIC at which 50 and 90% of the isolates are inhibited, respectively. MICs are expressed as mg/l  
S (%), per cent susceptible determined using NCCLS interpretive criteria

MIC, minimal inhibitory concentration

MRSA, methicillin resistant *Staphylococcus aureus*

MRCoNS, methicillin resistant coagulase negative staphylococci

MRSE, methicillin resistant *Staphylococcus epidermidis*

MRSH, methicillin resistant *Staphylococcus haemolyticus*

VREF, vancomycin-resistant *Enterococcus faecium*

2.0 mg/l and also presented similar activities to quinupristin/dalfopristin. None of the VREF isolates were resistant to linezolid, although a small percentage (5/96, 5.2%) of isolates displayed linezolid intermediate susceptibility (MICs= two isolates 3.0 and three isolates 4.0 mg/l). Among the five VREF isolates intermediate for linezolid, two were isolated from blood (MICs= 3.0 and 4.0 mg/l) and three from rectal swab cultures (MICs= one isolate 3.0 and two isolates 4.0 mg/l) of patients. Overall susceptibility of 96 VREF was 94.8 per cent, and there was no statistically significant difference between susceptibilities of isolates from blood (20/22, 90.9%) (percentage of resistant isolates 9.1%; 95% CI 0-2.1%) and rectal swab (71/74, 95.9%) (percentage of resistant isolates 4.1%; 95% CI 0-8.6%) cultures for linezolid. Percentage of VREF isolates susceptible to quinupristin/dalfopristin was 94.8 per cent and five isolates were found to be intermediate (MICs= 2.0 mg/l). One of these was from blood and other four were from rectal swab cultures and there was no statistically significant difference between the two groups (21/22, 95.4%, 70/74, 94.6%) (percentage of

resistant isolates 4.6%; 95% CI 0-9.5% and 5.4%; 95% CI, 0.25-10.5% respectively) for quinupristin/dalfopristin susceptibilities. Intermediate susceptibility to linezolid and quinupristin/dalfopristin belonged to different isolates.

## Discussion

Though E test is not approved by the NCCLS, there are numerous studies which showed that E test, disk diffusion and broth microdilution methods were comparable in accuracy for the susceptibility testing of MRSA and VRE against linezolid and quinupristin/dalfopristin<sup>17,18</sup>. We therefore used E test in this study to test *in vitro* susceptibilities of MDR Gram-positive cocci against quinupristin/dalfopristin and linezolid.

Linezolid has activity against most of the Gram-positive bacteria at 1.0-4.0 mg/l but Gram-negative bacteria are resistant owing to endogenous efflux<sup>1,6,19,20</sup>. Although MIC<sub>90</sub> values varied between species, resistance to linezolid was not observed in any MRSA and MRCoNS isolates. However, *S.*

*epidermidis* and *S. haemolyticus* showed similar susceptibility pattern for linezolid. Earlier studies have shown almost 100 per cent susceptibility for linezolid among staphylococci, enterococci, *S. pyogenes* and pneumococci<sup>1,7,18-21</sup>. Only two linezolid-resistant MRSA clinical isolates have been reported<sup>22,23</sup>. Since this agent is not yet available in Turkey, resistance due to prior exposure to linezolid is not expected, and in our study all of the MRSA and MRCoNS isolates were found to be susceptible to this agent.

Linezolid is shown to be highly active against enterococci including VRE<sup>7,17,19-21,24</sup>. However, resistance has been encountered especially in VREF isolated from patients who were treated with linezolid, and nosocomial spread of linezolid resistant strains has been documented<sup>25-27</sup>. Two such studies were reported from Greece<sup>28,29</sup>. Although the great majority of linezolid-resistant VREF infections reported to date have occurred in patients treated with linezolid, two reports have described patients without prior exposure to linezolid<sup>30,31</sup>. We found no linezolid-resistant VREF isolates in our study, possibly due to inavailability of this agent in our country. However, 5.2 per cent intermediate susceptibility rate seen must be taken into consideration since linezolid-resistant VREF isolates from patients without prior exposure to linezolid have been reported.

Quinupristin/dalfopristin is a mixture of quinupristin and dalfopristin, which are semisynthetic antibiotics of streptogramin groups B and A, respectively<sup>3</sup>. Studies reported that almost all isolates of MRSA and MRCoNS were susceptible<sup>1,2,5,18,19</sup>. In our study, the MICs for quinupristin/dalfopristin among staphylococci were low. There were no resistant isolates and only two MRCoNS isolates and three MRSA strains were intermediate. These results were similar to earlier studies. The resistance rates amongst the three most commonly occurring species, *S. epidermidis*, *S. haemolyticus* and *S. hominis* were reported to < 1.0 per cent<sup>2,32,33</sup>.

Enterococci have varying susceptibility to quinupristin/dalfopristin. Because of an efflux pump conferring resistance to dalfopristin appears to be intrinsic in this species, *E. faecalis* are generally the least susceptible<sup>1,34</sup>. However, most of the VREF

isolates were found to be highly susceptible to this agent<sup>1,2,5,19,33</sup>. Higher rates of resistance among VREF and other bacterial species were reported from Taiwan<sup>2</sup>. In the present study, we found that the 94.8 per cent of VREF isolates were susceptible to quinupristin/dalfopristin. There were no resistant isolates and only five strains found to be intermediate. In the present study, VREF isolates showing intermediate susceptibility to linezolid and quinupristin/dalfopristin were different from each other. In tertiary medical centers that have critically ill patients at high risk for VRE infection or colonization, it is recommended that periodic cultures of rectal swabs of such patients should be done to detect the presence of VRE and antimicrobial susceptibility surveillance should be performed even though VRE infections have not been identified clinically<sup>35</sup>.

Infections due to Gram-positive cocci are becoming increasingly more difficult to treat because of changes in the frequencies of isolation, distribution in the population, and their antibiotic resistance in Turkey and other parts of world<sup>36,37</sup>. The results demonstrated that the new streptogramin quinupristin/dalfopristin, and the oxazolidinone linezolid, have good *in vitro* activity against MDR Gram-positive cocci. These drugs are promising therapeutic options in an era of rapidly growing antibiotic resistance.

## References

1. Eliopoulos GM. Quinupristin-dalfopristin and linezolid: evidence and opinion. *Clin Infect Dis* 2003; 36 : 473-81.
2. Luh KT, Hsueh PR, Teng LJ, Pan HJ, Chen YC, Lu JJ, *et al.* Quinupristin-dalfopristin resistance among Gram-positive bacteria in Taiwan. *Antimicrob Agents Chemother* 2000; 44 : 3374-80.
3. Blondeau JM, Sanche SE. Quinupristin/dalfopristin. *Expert Opin Pharmacother* 2002; 3 :1341-64.
4. Lamb HM, Figgitt DP, Faulds D. Quinupristin/dalfopristin: a review of its use in the management of serious Gram-positive infections. *Drugs* 1999; 58 :1061-97.
5. Eiff C, Peters G. Comparative *in vitro* activities of moxifloxacin, trovafloxacin, quinupristin/dalfopristin and linezolid against staphylococci. *J Antimicrob Chemother* 1999; 43 : 569-73.

6. Livermore DM. Linezolid *in vitro*: mechanism and antibacterial spectrum. *J Antimicrob Chemother* 2003; 51(Suppl 2) : 9-16.
7. Ballow CH, Jones RN, Biedenbach DJ, the North American ZAPS Research Group. A multicenter evaluation of linezolid antimicrobial activity in North America. *Diagn Microbiol Infect Dis* 2002; 43 : 75-83.
8. Nathwani D. Impact of methicillin-resistant *Staphylococcus aureus* infections on key health economic outcomes: does reducing the length of hospital stay matter? *J Antimicrob Chemother* 2003; 51 (Suppl 2) : 37-44.
9. Hayden MK. Insights into the epidemiology and control of infection with vancomycin-resistant enterococci. *Clin Infect Dis* 2000; 31 : 1058-65.
10. Maschmeyer G, Noskin GA, Ribaud P, Sepkowitz KA. Changing patterns of infections and antimicrobial susceptibilities. *Oncology* 2000; 14 (Suppl 6) : 9-16.
11. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. The gram positive cocci: Part I: Staphylococci and related organisms. In: Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC, editors. *Color atlas and textbook of diagnostic microbiology*, 5th ed. New York: Lippincott; 1997 p. 539-76.
12. Facklam RR, Collins MD. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. *J Clin Microbiol* 1989; 27 : 731-4.
13. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing: twelfth informational supplement. NCCLS document M100-S12. Wayne, PA: National Committee for Clinical Laboratory Standards; 2002.
14. Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. *J Clin Microbiol* 1995; 33 : 24-7.
15. Tenover FC, Biddle JW, Lancaster MV. Increasing resistance to vancomycin and other glycopeptides in *Staphylococcus aureus*. *Emerg Infect Dis* 2001; 7 : 327-32.
16. Critchley IA, Draghi DC, Sahn DF, Thornsberry C, Jones ME, Karlowsky JA. Activity of daptomycin against susceptible and multidrug-resistant Gram-positive pathogens collected in the SECURE study (Europe) during 2000-2001. *J Antimicrob Chemother* 2003; 51 : 639-49.
17. Jorgensen JH, Crawford SA, Kelly CC, Patterson JE. *In vitro* activity of daptomycin against vancomycin-resistant enterococci of various Van types and comparison of susceptibility testing methods. *Antimicrob Agents Chemother* 2003; 47 : 3760-3.
18. Abb J. *In vitro* activity of linezolid, quinupristin-dalfopristin, vancomycin, teicoplanin, moxifloxacin and mupirocin against methicillin-resistant *Staphylococcus aureus*: comparative evaluation by the E test and a broth microdilution method. *Diagn Microbiol Infect Dis* 2002; 43 : 319-21.
19. Rybak MJ, Hershberger E, Moldovan T, Grucz RG. *In vitro* activities of daptomycin, vancomycin, linezolid, and quinupristin-dalfopristin against staphylococci and enterococci, including vancomycin-intermediate and -resistant strains. *Antimicrob Agents Chemother* 2000; 44 : 1062-6.
20. Szczypa K, Betlejewska K, Nowak K, Hryniewicz K. *In vitro* activity of linezolid against Gram-positive cocci isolated in Poland. *J Antimicrob Chemother* 2001; 48 : 932-5.
21. Sader HS, Gales AC, Jones RN. Antimicrobial activity of linezolid against Gram-positive cocci isolated in Brazil. *Braz J Infect Dis* 2001; 5 : 171-6.
22. Tsiodras S, Gold HS, Sakoulas G, Eliopoulos GM, Wennersten C, Venkataraman L, *et al*. Linezolid resistance in a clinical isolate of *Staphylococcus aureus*. *Lancet* 2001; 358 : 207-8.
23. Wilson P, Andrews JA, Charlesworth R, Walesy R, Singer M, Farrell DJ, *et al*. Linezolid resistance in clinical isolates of *Staphylococcus aureus*. *J Antimicrob Chemother* 2003; 51 : 186-8.
24. Henwood CJ, Livermore DM, Johnson AP, James D, Warner M, Gardiner A. The Linezolid Study Group. Susceptibility of Gram-positive cocci from 25 UK hospitals to antimicrobial agents including linezolid. *J Antimicrob Chemother* 2000; 46 : 931-40.
25. Herrero IA, Issa NC, Patel R. Nosocomial spread of linezolid-resistant, vancomycin-resistant *Enterococcus faecium*. *N Engl J Med* 2002; 346 : 867-9.
26. Auckland C, Teare L, Cooke F, Kaufmann ME, Warner M, Jones G, *et al*. Linezolid-resistant enterococci: report of the first isolates in the United Kingdom. *J Antimicrob Chemother* 2002; 50 : 743-6.

27. Johnson AP, Tysall L, Stockdale M, Woodford N, Kaufmann ME, Warner M, *et al.* Emerging linezolid-resistant *Enterococcus faecalis* and *Enterococcus faecium* isolated from two Austrian patients in the same intensive care unit. *Eur J Clin Microbiol Infect Dis* 2002; 21 : 751-4.
28. Metallidis S, Chatzidimitriou M, Nikolaidis P, Tsona A, Bisiklis A, Kollaras P, *et al.* Comparative *in vitro* activity of linezolid and five other antimicrobials against nosocomial isolates of methicillin-resistant *Staphylococcus aureus*, methicillin-resistant *Staphylococcus epidermidis* and vancomycin-resistant *Enterococcus faecium*. *J Chemother* 2003; 15 : 442-8.
29. Bersos Z, Maniati M, Kontos F, Petinaki E, Maniatis AN. First report of a linezolid-resistant vancomycin-resistant *Enterococcus faecium* strain in Greece. *J Antimicrob Chemother* 2004; 53 : 685-6.
30. Jones RN, Della-Latta P, Lee LV, Biedenbach DJ. Linezolid-resistant *Enterococcus faecium* isolated from a patient without prior exposure to an oxazolidinone: report from the SENTRY Antimicrobial Surveillance Program. *Diagn Microbiol Infect Dis* 2002; 42 : 137-9.
31. Rahim S, Pillai SK, Gold HS, Venkataraman L, Inghima K, Press RA. Linezolid-resistant, vancomycin-resistant *Enterococcus faecium* infection in patients without prior exposure to linezolid. *Clin Infect Dis* 2003; 36 : 146-8.
32. John MA, Pletch C, Hussain Z. *In vitro* activity of quinupristin/dalfopristin, linezolid, telithromycin and comparator antimicrobial agents against 13 species of coagulase-negative staphylococci. *J Antimicrob Chemother* 2002; 50 : 933-8.
33. Jones RN, Ballow C, Biedenbach DJ, Deinhart JA, Schentag J. Antimicrobial activity of quinpristin-dalfopristin (RP 59500, Synercid®) tested against 28,000 recent clinical isolates from 200 medical centers in the United States and Canada. *Diagn Microbiol Infect Dis* 1998; 30 : 437-51.
34. Eliopoulos GM, Wennersten CB, Gold HS, Schülin T, Souli M, Farris MG, *et al.* Characterization of vancomycin-resistant *Enterococcus faecium* isolates from the United States and their susceptibility *in vitro* to dalfopristin-quinupristin. *Antimicrob Agents Chemother* 1998; 42 : 1088-92.
35. Centers for Disease Control and Prevention. Preventing the Spread of Vancomycin Resistance - A Report from the Hospital Infection Control Practices Advisory Committee; Comment Period and Public Meeting Notice. Federal Register, May 7, 1994; 59 : 25758-63.
36. Schouten MA, Hoogkamp-Korstanje JAA, Meis JFG, Voss A. Prevalence of vancomycin-resistant enterococci in Europe. *Eur J Clin Microb Infect Dis* 2000; 19 : 816-22.
37. Colak D, Naas T, Gunseren F, Fortineau N, Ogunc D, Gultekin M, *et al.* First outbreak of vancomycin-resistant enterococci in a tertiary hospital in Turkey. *J Antimicrob Chemother* 2002; 50 : 397-401.

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